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Na, K and Ca Contents in Roots and Leaves of Three *Glycine* Species Differing in Response to NaCl Treatments

Wen-Yuan Kao(1,2*)

1. Institute of Ecology and Evolutionary Biology, National Taiwan University. 1, Roosevelt Rd., Sec. 4, Taipei 106, Taiwan.
2. Department of Life Science, National Taiwan University. 1, Roosevelt Rd., Sec. 4, Taipei 106, Taiwan.
** Corresponding author. Email: wykao@ntu.edu.tw*

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ABSTRACT: It has been shown that three wild soybean species, *Glycine soja*, *G. tomentella* and *G. tabacina*, in Taiwan had different response to root-zone salinity and differences in leaf accumulation of Na and K might be responsible for the response. To further understand the mechanisms in relation to the differences among species in leaf accumulation of Na, K and Ca, in this study I compared the distribution of Na, K, and Ca not only in leaves but also in roots and calculated the sum of the ion contents in leaf and root (= ion content in roots + ion content in leaves) of the three soybean species receiving four different levels of NaCl treatments, 0, 17, 51 and 85 mM. Analysis of contents of K and Na in leaves and roots revealed that *G. soja*, the most sensitive species, and *G. tomentella*, the most tolerance species had similar sum of root and leaf Na contents under the same NaCl treatment, however, the former had significantly high leaf Na content than the later. *G. tabacina*, the medium sensitive species, had the least sum of root Na and leaf Na contents and medium leaf Na content. In *G. soja*, increasing accumulation of Na in leaves with increasing treatment NaCl concentration might hamper the uptake of K in roots resulting in decreases in the sum of K contents in roots and leaves. There is no evidence that salinity could impair Ca uptake in the three wild soybean species. These results suggest that differential sensitivity to root-zone salinity among the three species is mainly due to their differential ability to control the allocation of salt away from leaves and to some extent restrict Na uptake at root level.

KEY WORDS: *Glycine*, growth, ion contents, salinity, wild soybean.

INTRODUCTION

Salinity is one of the environmental factors affecting plant growth and crop yield. Because of the increasing utilization of ground water, many areas devoted to agriculture are becoming salty and suffering reduction in productivity (Szabolcs, 1994). Soybean is classified as a glycophyte whose growth and development is generally limited by salinity. For example, it has been shown that the productivity of 20 soybean cultivars was reduced when the soil salinity exceeding 14 ds/m (Chang et al., 1994). Soybean is one of the important crops for human diets. With an increasing in food demanding from the growing human population, there is an urgent need to develop salt-tolerant soybean and other crops. To improve the ability of current crop variety to grow in saline soil, it is necessary to understand traits and mechanisms contributing to salt-tolerance in related cultivars or their wild-type relatives.

There are three wild soybean species in Taiwan, *G. soja* Sieb & Zucc., *G. tomentella* Hayata and *G. tabacina* (Labill.) Benth. Differences in their growth and physiological response to salinity have been reported among these three species, with *G. soja* being the most sensitive, *G. tabacina* intermediate, and *G. tomentella* the least sensitive (Kao et al., 2003; Kao et al., 2006). It has also been found that the three species differed in concentrations of Na and K in leaf tissue, and the differential accumulation of leaf Na and K might be responsible for the different sensitivity of the three wild soybeans. However, the mechanisms contributing to the differential concentrations of Na and K in leaf tissue of the three species has not been studied. It has also been shown that salinity can interfere with K and Ca nutrition (Rengel, 1992). In addition, it is know that not only the ability to control the allocation of salt, but the ability to control the entry salt into the plant also plays important role in plant tolerance to salinity stress (Cheeseman, 1988; Munns and Tester, 2008; Plett and Møller, 2010).

In this study, I expanded a previous study (Kao et al., 2006) by including the measurement of the major ion (K, Na and Ca) contents of roots of the three wild soybean species subjected to four NaCl treatments to further reveal the possible mechanism associated with the difference in concentrations of Na and K in leaf tissue of the three species. Specifically, I asked following question. Is the difference in concentrations of Na in leaf tissue of the three soybean species due to the differential ability to control the uptake of salt in the whole-plant or due to the differential ability to control the allocation of salt away from leaves?

MATERIALS AND METHODS

G. soja seeds were collected from Shihmen (25°02’ N, 120°30’ E) in northern Taiwan, *G. tomentella* from Hengchun (22°00’ N, 120°44’ E) in southern Taiwan, and *G. tabacina* from Penghu island (23°34’ N, 119°33’

E). Seeds were germinated in a Petri dish. After germination, seedlings with 2 trifoliates were transferred to 2L plastic pots, with one seedling per pot, filled with a mixture of vermiculite and soil (1:1 by volume). Plants were grown in a glasshouse under natural daylight, watered every day, and fertilized using inorganic fertilizer (N:P:K at a ratio of 20:20:20) once every 2 weeks. The salinity treatment began when plants were 4 weeks old. Instead of watering plants with different concentrations of NaCl, a modified method was used to avoid the accumulation of NaCl in the pots. The pots were immersed in a modified Hoagland's solution (Haines and Dunn, 1976), containing 0, 17, 51, 85 mM of NaCl, for two hours every day, with four replicates per species for each salinity treatment. Final NaCl treatment concentrations were reached gradually by increment of 17 mM every two days. Then, plants were subjected to the final treatment concentration for two months.

A trifoliate leaf from each plant was collected for ion content analysis after 1 month of salinity treatment. After 2 months of treatment, plants were harvested and separated into shoots (leaf and stem) and roots components, dried at 60 ℃ for 7 days and then weighted. Leaf and root components were grounded to powder for ion contents analysis.

Organic matter of leaves and roots was destroyed by combustion at a temperature of 470 ℃ for 16 hr in presence of air. The residue ash was then dissolved in a dilute acid (6M HCl) to bring the mineral elements into the solution (Lamber, 1976). The solution was filtered with Whatman 42 filter paper. Potassium and Na contents of the extract were subsequently analyzed with a flame photometer (Model 410, Corning, England). Calcium contents of the extract were analyzed by atomic absorption (Model 2380, Perkin-Elmer) (Isaac and Kerber, 1971).

All statistical tests were performed using the computer software SYSTAT (Statistical Solution Limited, Ireland). One way ANOVA was performed and means were compared by the least significant difference test. Significant levels are reported as $P < 0.05$. Though the result of statistic analysis was not presented in Figs. 2-4, it was addressed in the text.

RESULTS

Accumulated biomass

Among the treatments, *G. soja* and *G. tabacina* received 0 mM NaCl treatment accumulated the most biomass, their accumulated biomass was significantly reduced under 17 mM NaCl treatment (Fig. 1). In contrast, no significant difference was found in the accumulated biomass between 0 mM and 17 mM NaCl treated *G. tomentella*. For all three species, 51 and 85 mM treatments resulted in significantly less accumulated biomass than controls. However, the reduction was less in *G. tabacina* and *G. tomentella* than in *G. soja*.

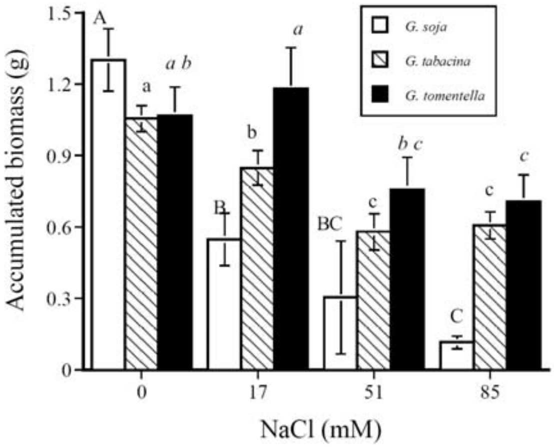

Fig. 1. Accumulated biomass of three soybean species, *G. soja* (open bar), *G. tabacina* (closed bar), and *G. tomentella* (hatch bar), exposed to four NaCl treatments for 2 months. Vertical bars represent 1 S.E. of treatment mean (n = 4). Values with different letters are significantly different (P<0.05) among NaCl treatments.

Three species received 0 mM of NaCl treatment (Fig. 1) accumulated similar amount of biomass after two months of treatment. Due to their differential response to increasing in concentration of NaCl treatment, at 17, 51 and 85 mM NaCl treatments, *G. tomentella* accumulated the most, the *G. tabacina* the medium and *G. soja* the least amount of biomass after two months of treatment.

Na, K and Ca contents

No significant difference was found in leaf Na contents among the control plants of the three species (Fig. 2) after one or two months of treatment. However, increases in root-zone salinity had different effect on leaf Na content of the three species. After being exposed to different concentration of NaCl for one month, *G. soja* received 51 and 85 mM NaCl treatments accumulated significantly more Na in leaf tissue compared to those at 0 and 17 mM NaCl treatments. In contrast, *G. tabacina* and *G. tomentella* received different NaCl treatments for one month showed similar Na content in their leaf tissue. Consequently, under 51 and 85 mM NaCl treatments for one month, leaves of *G. soja* had higher Na content than those of *G. tabacina* and *G. tomentella.*

After two months of treatment, compared to control plants *G. tabacina* and *G. tomentella* received 51 and 85 mM NaCl also showed increase in leaf Na content, however, the increase was less than *G. soja* received the same NaCl treatment (Fig. 2). Slight increase in the amount of Na content was found in roots of *G. soja* and *G. tabacina* received 17, 51 and 85 mM NaCl treatment. In contrast, supplemental of NaCl significantly increased

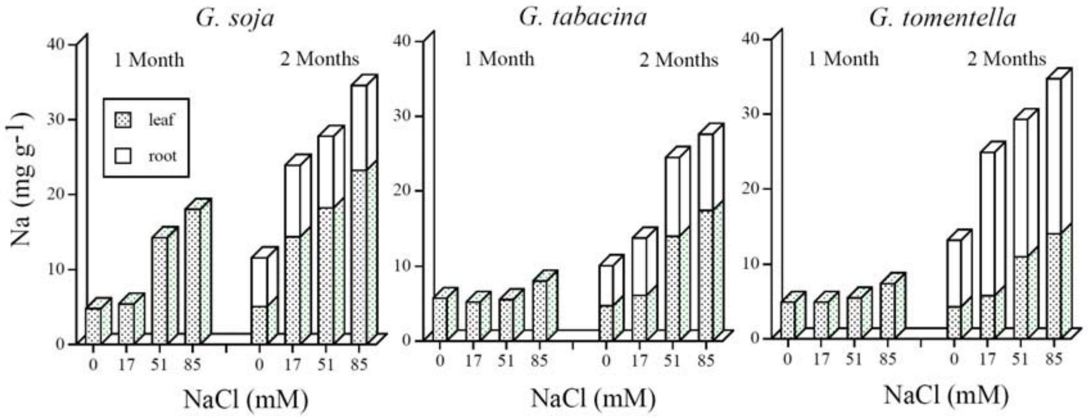

Fig. 2. The mean Na content in leaves (dotted bar, n = 4) and roots (open bar, n = 4) of three soybean species, *G. soja*, *G. tabacina*, and *G. tomentella*, exposed to different NaCl levels for one and two months.

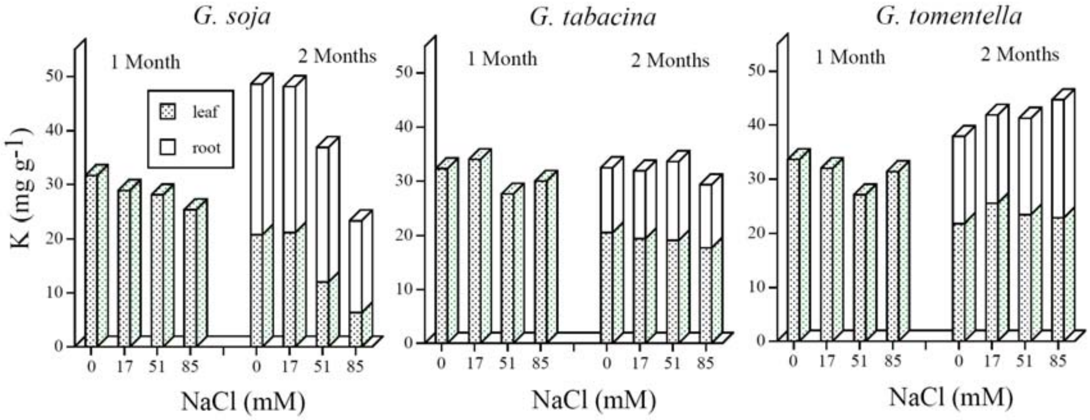

Fig. 3. The mean K content in leaves (dotted bar, n = 4) and roots (open bar, n = 4) of three soybean species, *G. soja*, *G. tabacina*, and *G. tomentella*, exposed to different NaCl levels for one and two months.

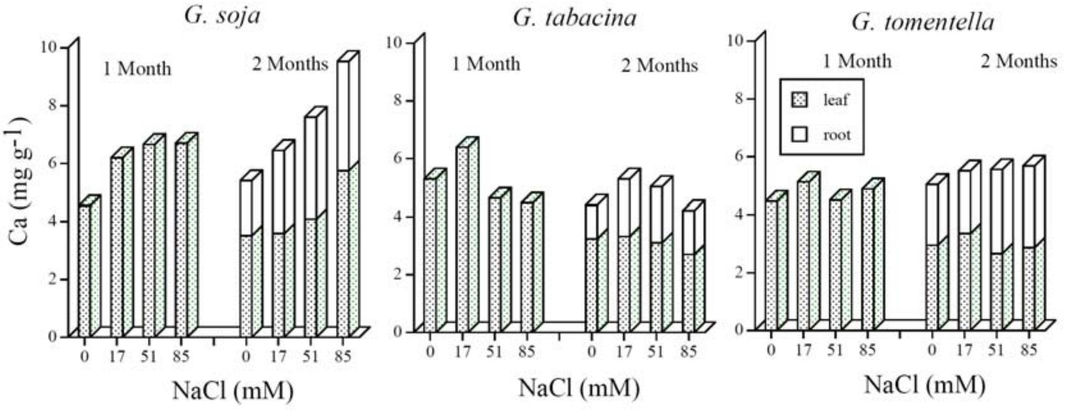

Fig. 4. The Ca content in leaves (dotted bar, n = 4) and roots (open bar, n = 4) of three soybean species, *G. soja*, *G. tabacina*, and *G. tomentella*, exposed to different NaCl levels for one and two months.

Na content in roots of *G. tomentella* in comparison to that in control plants. However, no significant difference was found in Na content among roots of 17, 51 and 85 mM treated *G. tomentella*.

The contents of Na between leaves and roots were also different among the three species (Fig. 2). No significant difference in Na content was found between shoots and roots of 0mM treated *G. soja*, as NaCl increased, leaves accumulated significantly more Na than roots. A similar pattern was found in 51 and 85 mM treated *G. tabacina*, however, the amount of Na content in leaves was significantly lower in *G. tabacnia* than in

G. soja compared at the same NaCl (17 to 85 mM) treatment. In *G. tomentella*, roots had significantly more Na content than leaves regardless of the treatment.

After two months of treatment, the sum of Na contents in roots and leaves (the sum of Na) of the three species all increased with increasing NaCl. However, the amount of increment differed among the three species, with *G soja* and *G. tomentella* showing similar amount of increment while less increment in *G. tabacina*. Consequently, at 85 mM NaCl treatment, *G. tabacina* had the least, while *G. soja* and *G. tomentella* had similar sum of Na content in roots and leaves.

Compared 0mM treated plants, *G. soja* had the highest, *G. tomentella* the intermediate, and *G. tabacina* the least sum of K content in roots and leaves (sum of K content) (Fig. 3). In response to increasing in NaCl, no significant change in leaf K content or in root K content was found in *G. tomentella* and *G. tabacina,* in contrast, a dramatic decline in the sum of K content was measured in 51mM and 85mM treated *G. soja*. Irrespective of treatments, leaves of *G. tomentella* and *G. tabacina* always had higher K than their roots, in addition, the distribution of K between roots and leaves of these two species were not affected by NaCl treatments. Roots (or leaves) of 0mM and 17mM treated *G. soja* contained similar amount of K. Increasing in treatment NaCl concentration resulted in significantly lower K contents in roots of 85mM and in leaves of 51mM and 85mM treated *G. soja* plants. As a result, the sum of K contents was significantly lower in 51mM and 85mM than in 17mM and 0mM treated *G. soja*.

Increasing in treatment NaCl concentration resulted in significantly increasing in the sum of Ca content in root and leaf in *G. soja* plants, the increment was mainly due to increases in root Ca (Fig. 4). In response to increasing in NaCl, no significant change in leaf Ca content or in root Ca content was found in *G. tomentella* and *G. tabacina*. Thus, the sum of Ca content in *G. tomentella* and *G. tabacina* remained similar among the treatments.

DISCUSSION

The growth or the accumulation of biomass is often used as a parameter to evaluate tolerance (Cheeseman, 1988). Based on their response in accumulated biomass to the increasing levels of NaCl treament, *G. soja* was the most sensitive, *G. tabacina* the intermediate, and *G. tomentella* the least sensitive species to salinity (Fig. 1) among the three species. In a previous study, the different sensitivity of the three wild soybeans in response to increases in root-zone NaCl concentration was found to be related to the differential accumulation of leaf Na and K (Kao et al., 2006). Extension from the previous study, result from this study further revealed that the three species not only accumulated different amount of Na and K in leaves, they also differed in the accumulation of these ions in roots, as a result, the distribution of ions between root and leaf also differed among the three species (Fig. 2). The results provided possible mechanisms for differential accumulation of leaf Na and K among the three wild soybean species, hence, their different ability to tolerant salinity.

Compared the Na distribution between tissue components of the most sensitive (*G. soja*) and the most tolerant species (*G. tomentella*) among the three species revealed that their sum of Na contents in leaves and roots were similar under the same NaCl treatment, however, the distribution of Na between leaves and roots differed between them, with the tolerant species accumulating more in roots than in leaves and vice versa in the sensitive species. In comparison to *G. tomentella, G. tabacina* had less sum of Na content but more leaf Na content, however, it is not more tolerant to salinity. These results suggested that Na exclusion from the shoot played an important role in determining the tolerant ability among the three wild soybean species in response to increases in root-zone NaCl concentration. Na exclusion from the shoot has also been reported as an important mechanism in salinity tolerance in crops such as durum wheat (Munns and James, 2003). Genes belongs to the HKT or SOS1 family in *Arabidopsis* and other species have been implicated in controlling Na movement throughout the plant (Tester and Davenport, 2003; Apse and Blumwald, 2007; Munns amd Tester, 2008). Recently, Jha et al. (2009) reported that among *Arabidopsis* ecotypes variation in shoot Na accumulation was linked to differences in the natural expression levels of Na transporter in the roots. To further understand the differential ability in Na exclusion in the three wild soybean species, sodium transporter of roots of the three species will be studied in the future.

Structure adaptations, including salt gland, could play significant role in reducing salt content in leaves. All three soybean species have hairs on their leaf surface (Huang et al., 1984). Salt gland-like structure was found in leaf and stems of *G. soja* collected from the estuary of the Yellow River in China (Lu, 1998), however, its function was not studied. Whether the structure and hairs on leaf surface of the other two species have the capacity for salt secretion needs to be further investigated.

Though *G. tabacina* seemed to have less ability than *G. tomentella* in exclusion of Na from leaves, it seemed to have greater ability than the other two species to restrict Na uptake at root level (Fig. 2). This ability might result in less Na accumulated in leaves and hence contribute to its more tolerant to salinity than *G. soja*. The lower transpiration rate in G. *tomentella* (Kao et al., 2003) might contribute to its lower uptake rate of Na and K into roots.

The dramatic decline in the sum of K content in roots and leaves and in the K content of leaves in 51 and 85 mM NaCl treated *G. soja* (Fig. 3) suggested that accumulation of Na in leaves (Fig. 2) might hamper the uptake of K in roots and preventing the distribution of K from roots to shoots. The result explained the high Na/K ratio at leaf level found in high NaCl treated *G. soja*.

Huang and Redmann (1995) reported that salinity-induced symptoms in cultivated barley may be the results of a Ca deficiency. In this study, no significant effect of salinity on Ca content in roots and leaves was found in *G. tomentella* and *G. tabacina*. In contrast, there were increases in the sum of Ca content in roots and leaves of *G. soja*, the most sensitive species, in response to salinity treatments. Accordingly, salinity-induced impairing of Ca uptake was not evident in this study.

In conclusion, differences among the three soybean species in leaf accumulation of Na might be due to their differential ability to control the allocation of Na salt away from leaves and the control of salt entry into the whole-plant. The accumulation of Na might hamper the uptake of K in roots and preventing the distribution of K from roots to shoots.

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對鹽處理有不同反應的三種野生大豆根和葉部鈉、鉀和鈣的含量比較

高文媛[1,2*]

1. 臺灣大學生態學與演化生物學研究所，106 台北市羅斯福路 4 段 1 號，臺灣。
2. 臺灣大學生命科學系，106 台北市羅斯福路 4 段 1 號，臺灣。
* 通信作者。*Email: wykao@ntu.edu.tw*

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摘要：先前研究發現臺灣三種野生大豆 (*Glycine soja, G. tomentella* 和 *G. tabacina*) 對土壤鹽分濃度增加有不同程度的反應，*G. tomentella* 耐受度最好，*G. tabacina* 其次，*G. soja* 最不耐，且其敏感度和葉部組織內鈉和鉀的累積量有相關 (Kao et al., 2006)。為了瞭解是什麼機制導致這三種野生大豆部葉部組織內有不同的鈉、鉀和鈣的累積量，本文延伸先前研究，進一步分析這三種野生大豆在四種不同鹽分濃度 (0、17、51 和 85 mM) 處理下其鈉、鉀和鈣分別在根部、葉部的含量、以及計算根和葉含量總合 (根部加上葉部的含量) 變化。結果在相同鹽分濃度處理下 *G. soja* 和 *G. tomentella* 有類似的根和葉鈉含量總合，然而前者葉鈉含量顯著高於根鈉含量，後者則有相反的反應。比較 *G. tabacina* 和 *G. tomentella* 則發現雖然前者根部和葉部鈉含量總合顯著低於後者，然而其葉鈉含量比後者高。當較多的鈉累積在 *G. soja* 的葉部時，可能降低其根對鉀的吸收，以至於在 51 和 85 mM 處理下，其根部和葉部鉀含量總合顯著減少。鹽濃度增加並沒有減少大豆植株根部和葉部的鈣含量。綜合上述,大豆耐鹽逆境的機制應該和其是否有能力減低葉部鈉含量(主要機制)以及減少根部對鈉的吸收(次要機制)有關。

關鍵詞：*Glycine*、生長、離子含量、鹽逆境、野生大豆。